EXHIBIT 2

NIGHT CARE CREAM "IMMORTELLE"
Anti radical properties

1. DISPLAY OF THE ANTI FREE RADICAL PROTECTION ACCREDITED TO NIGHT CARE *IMMORTELLE'S* ACTION :

This property has been shown by MDA dosage which is a lipoperoxydation index.

In order to show this property, we used Skin Ethic® (Nice, France), an *in vitro* reconstituted epidermis model which allows the dosage of different biochemical markers.

BIBLIOGRAPHIC REFERENCES :

CULTURE PROCEDURE :

Human origin keratinocytes are inoculated on 0,63 cm^2 or 4 cm^2 polycarbonate filters in a defined media (modified MCDB 153) and supplemented.

Cells are cultured during 14 days at the air/liquid interface. Culture media is changed every two days.

Thus formed epidermis has been used for the study starting the 17th day of culture.

STUDIED PRODUCT :

NIGHT CARE *IMMORTELLE* has been applied on the surface of each epidermis, at the rate of 2 mg/cm^2.

2. DOSAGE METHODS

Epidermis have been treated (24h), washed (iced NaCl 0,9%) and collected.
The tissues have been homogenised in a Tris HCl buffer volume (1ml), 20 mM, pH 7,4 at 4°C with a glass polygrinder.
Each homogenate is taken and centrifuged at 300g and 4°C during 10mn.
Supernatant is taken and used for our dosage (500 µl).
50 µl BHT (2% in absolute ethanol) are added in each supernatant.

2.1 TBARS (THIOBARBITURIC ACID-REACTIVE SUBSTANCES) DOSAGE :

- The complexation reaction is initiated by 1 ml of TBA 0,375% (in 0,25M HCl containing 15% Trichloracetic acid).
- 1 hour incubation in a 80°C water bath.
- The reaction is stopped by a brutal cooling in ice (20 minutes).
- Extraction of the molecules that reacted with the TBAR is done by adding 300 µl butan-1-ol strongly shaked with a vortex.
- A 10 min. 3000 rpm centrifugation is necessary to get the organic phase.
- Each butanolic phase is collected and transferred in the wells of a microtitration black plate.
- Reading by spectrofluorometry (*FLUOstar, BMG*) at 515 nm excitation wavelength, the reading being done at 553 nm.

- The samples are compared to a standard 1,1,3,3- tetrametoxypropan (*Malonaldehyde bis [dimathyl acetal], Sigma #T-1642*) scale, to determine MDA concentration in the solution.

2.2 PROTEIN DOSAGE :

For each sample we determine a protein concentration of the collected tissue's content.
The procedure consists of associating to this content (50 µl) a mix (100µl) of a bicinchoninic acid (BCA) solution and of a Copper sulfate solution. Cu++ ions of this mixture are reduced to Cu+ in presence of proteins. These ions chellate with 2 BCA molecules.
Samples are read with a spectrophotometer (*Dynatech reader*) at 550 nm and compared to a Bovine Serum Albumine (BSA) standard scale to determine the total protein concentration (in BSA equivalents) of the solution.

3. CALCULATION OF THE MDA CONCENTRATION :

Final results are expressed in : **MDA nM / protein mg** .

4. RESULTS (GRAPHIC) :

In the selected experimental conditions , the **NIGHT CARE *IMMORTELLE*** product applied on reconstituted epiderms in a defined media, had a significant anti free radical activity.

Indeed, we could notice an inhibitory action on lipoperoxydation **(~ 50% protection)**.

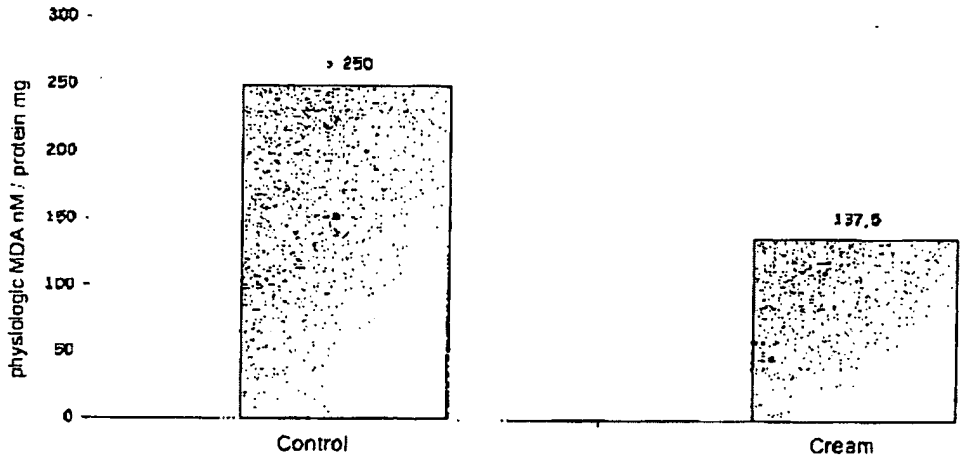
OCCITANE

« IMMORTELLE »

CREAM

Anti-radicalair activity

Physiological lipoperoxydation measure by MDA* dosage



* Fluorescence measure of malonaldehyde (MDA)

Dosage after 24 hours application on reconstituted epiderm